ACTIVATED GTPASE-BASED ASSAYS AND KITS FOR THE DIAGNOSIS OF SEPSIS AND OTHER INFECTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Patent Application Ser. No. 61/941,604, entitled "Rapid, Effector-Based, Flow-Cytometry Assay for Activated GTPases", and filed 19 Feb. 2014. The complete contents of this provisional patent application are hereby incorporated by reference in their entirety.

STATEMENT REGARDING FEDERAL FUNDING

[0002] This invention was made with government support under Grant No. 1R21NS066435 awarded by the National Institute of Neurological Disorders and Stroke (NINDS); Grant Nos. R03A1082130, R03AI092130, R21NS066429 and 1P50GM085273 awarded by the National Institute of Health (NIH); and Grant No. MCB0956027 awarded by the National Science Foundation (NSF). Consequently, the government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] In one embodiment, the invention provides a method of diagnosing a bacterial infection (sepsis) in a subject by using multiplexed flow cytometry to detect and measure the level of active GTPase enrichment caused by bacterial infection. Related kits are also provided.

[0004] In a preferred embodiment, the invention provides point of care activated GTPase-based diagnostic methods and related kits for determining an early stage sepsis, especially in a hospital setting.

[0005] More specifically, certain embodiments provide a rapid assay for measuring the cellular activity of small GTPases in response to a specific stimulus. Effector functionalized beads are used to quantify in parallel multiple, GTPbound GTPases in the same cell lysate by flow cytometry. In particular biologically relevant examples, different Ras (HRas, Rap1), Rho (Rac1, Cdc41, RhoA) and Rab (Rab 5) family GTPases are shown for the first time to be involved in a concerted signaling cascade downstream of receptor ligation by Sin Nombre hantavirus. In another setting, the preclinical onset of sepsis was manifested by the enrichment of active GTPases (Rho, Rap1 or Rac1) measured in serial plasma samples taken from trauma patients who were clinically diagnosed with bacterial infection.

BACKGROUND OF THE INVENTION

[0006] The Ras superfamily of small GTPases is comprised of five major groups: Ras, Rho, Rab, Arf and Ran that regulate many aspects of cell behavior. The Ras (e.g. H-Ras, K-Ras, R-Ras and Rap 1) and Rho subfamily (e.g. Cdc42, Rac1 and RhoA) of GTPases synergistically regulate signaling pathways that originate from extracellular stimuli, to yield overlapping sets of cellular phenotypes, such as proliferation, differentiation, and remodeling of the cytoskeleton. The GTPases function by cycling between active GTP-bound and inactive GDP-bound states. Guanine nucleotide-exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide-dissociation inhibitors (GDIs) (Guilluy et al., 2011; Jaffe and Hall, 2005) control the activity of the GTPases. GEFs activate Rho proteins by catalyzing the exchange of GDP for GTP, while GAPs inactivate the proteins by stimulating intrinsic GTPase activity. GDI inhibits the activation of Rho GTPases by sequestering them in the cytosol away from membranes. Activated GTPases interact with specific downstream-effector proteins to yield definite physiological responses in response to the upstream stimuli. Ras and Rho family GTPases function as components of a broader signaling network and are interconnected across overlapping signaling pathways that involve positive and negative feedback loops.

[0007] The superfamily of GTPases has numerous cellular effects that are dysregulated in disease. Ras (35 members) primarily involved in signaling and cancer. Rho (23 members) GTPases are primarily involved in cell motility, infection and cancer among others. Rab (70 members) GTPases are primarily involved in intracellular transport, cancer, infectious disease, genetic disease and downstream growth factor signaling Ran (1 member) nuclear import, cellular differentiation, Arf (30 members) intracellular transport, infectious disease, human ciliopathies and retinopathies. The dysregulation of these systems can be measured as an increase in the enrichment of active GTPases caused by factors in patient samples. Active GTPases preferentially bind to specific cognate effector molecules that are immobilized on beads, thus providing evidence as to the dysregulation of the systems involved.

[0008] The interactions of viruses and host cells is known to elicit the activation of multiple GTPases to promote the cytoskeletal remodeling required for breaching inter- and intracellular cellular barriers to infection as well as intracellular trafficking of internalized virions to allow replication. Most studies investigating the role of GTPases in viral interactions with host cells use traditional methods of active GTPase pull-down and detection by Western blot, which are slow, labor intensive and require large amounts of starting material. Newer, commercially available plate-based effector binding assays for detecting activated GTPases known as GLISA (Cytoskeleton, Inc.) require less material than western blot based assays, yet are still labor intensive; requiring freezing of aliquots, protein assays to ensure linearity and numerous binding and washing steps. Accordingly, most studies tend to focus on a limited subset of GTPases, which presents significant limitations when one wants to examine the broader spectrum of cell signaling space impinged upon by viral activity. Based on these considerations, we have developed a rapid, and quantitative flow cytometry-compatible, bead-based effector binding assay to analyze, in parallel, multiple GTPases that are activated in a single virus-infected cell sample. Sepsis is a disease that now affects more than 900,000 patients with an estimated mortality rate of 30% in the US.[22-25A] Annual costs are estimated to exceed $20 billion.[26A] Severe trauma patients who survive the initial injury are at risk of developing sepsis syndrome and multiple organ failure.[24A, 25A, 27A] Systemic microvascular leakage, most likely due to the release of inflammatory, coagulation and fibrinolysis factors, is a signature of sepsis in trauma patients.[22A] An improved understanding of the clinical mechanisms of sepsis, including the roles of pathogens, sites of injury and patient heterogeneity, is urgently needed to enable better prevention, diagnosis, and treatment.[22A] The goal of this project is to address the need for timely and accurate differential diagnosis of sepsis and SIRS due to sterile inflammation. The pathophysiology of sepsis